warts cell differentiation is impaired to a much lesser degree [13]. Thus the greater disturbance of cells and tissue may be the result of the higher production of mature virions.

In condylomata acuminata the detection of virions by electron microscopy is a rare event. This finding could be correlated with the hypothesis of defective virus maturation in spite of the abundance of viral DNA that is present in normal genomic length [3]. Possibly the different virus DNAs code different ways of virus maturation.

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REFERENCES

1. Coggin JR Jr, zur Hausen H: Workshop on papilloma-virus and cancer. Cancer Res 39:545–546, 1979
2. Gissmann L, Pfister H, zur Hausen H: Human papilloma viruses (HPV): characterization of four different isolates. Virology 76:569–580, 1977
3. Gissmann L, zur Hausen H: Partial characterization of viral DNA from human genital warts (condylomata acuminata). Int J Cancer 25:605–609, 1982
4. Orth G, Favre M, Croissant O: Characterization of a new type of human papilloma-virus that causes skin warts. J Virol 24:108–120, 1977
5. Orth G, Jablonska S, Favre M, Croissant O, Jarzabek-Chorzelska M, Rzesa, G: Characterization of two types of human papillomaviruses in lesions of epidermodysplasia verruciformis. Proc Natl Acad Sci USA 75:1537–1541, 1978
6. Orth G, Jablonska S, Favre M, Croissant O, Obalek S, Jarzabek-Chorzelska M, Jibard N: Identification of papillomaviruses in butchers' warts. J Invest Dermatol 76:97–102, 1981
7. Pfister H, Nürnberger F, Gissmann L, zur Hausen H: Characterization of a human papillomavirus from epidermodysplasia verruciformis lesions of a patient from Upper-Volta. Int J Cancer 27:645–650, 1981
8. Dvoretzky I, Douglas RL: Infections by human papilloma-virus (warts). Am J Dermatopathol 4:85–89, 1982
9. Gissmann L: Vergleichende Charakterisierung der Desoxyribonucleinsäuren menschlicher Papillomviren (HPV). Dissertation, Erlangen, 1977
10. Gissmann L, Diehl V, SchultzCoulon H-J, zur Hausen H: Molecular cloning and characterization of human papillomavirus DNA derived from a laryngeal papilloma. J Virol, 44:393–400, 1982
11. Fresen KO, Cho MS, Gissmann L, zur Hausen H: NC 37-R1 Epstein-Barr virus (EBV): a possible recombinant between intracellular NC 37 viral DNA and superinfecting P3 HR-1 EBV. Intervirology 12:303–310, 1979
12. zur Hausen H, Meinhof W, Scheiber W, Bornkamm GW: Attempts to detect virus-specific DNA in human tumors. I. Nucleic acid hybridization with complementary RNA of human wart virus. Int J Cancer 13:650–656, 1974
13. Grussendorf E-I: Lichtmikroskopische Untersuchungen an typisierten Viruswarzen (HPV-1 und HPV-4). Arch Dermatol Res 268:141–148, 1980

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Papillomavirus in Cervical Condylomas With and Without Associated Cervical Intraepithelial Neoplasia

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The aim of this study was to analyze the cytohistologic features of cervical condylomas with respect to the presence of associated cervical intraepithelial neoplasia (CIN) and to evaluate whether or not the prevalence of virus antigen, as detected by immunologic staining with peroxidase-antiperoxidase technique, varies with the histologic appearance of the lesions. In a series of 94 histologically confirmed condylomas of the cervix, almost half (43) had features of CIN grades 1 and 2, corresponding to mild and moderate dysplasia. The prevalence of papillomavirus antigen decreased markedly as the features of associated dysplasia became more severe. The antigen prevalence was 82% in pure condylomas, 32% in condylomas with CIN 1, and 0% in condylomas with CIN 2.

The evidence that virus production decreased as the lesion became more severe does not preclude papillomavirus etiology for the CIN lesions. Cells transformed by papillomaviruses may be expected to cease production of virus particles even as they continue to harbor the viral genome.

Epidemiologic studies have shown that squamous carcinoma of the female genital tract is a sexually transmitted disease [1]. The search for the etiology of these cancers has, in recent years, focused primarily on viral agents, in particular herpes simplex 2. However, the frequent association of genital warts with vulvar and penile carcinomas [2,3] and the confirmation of the papillomavirus etiology of a flat cervical lesion, which had previously often been named as a mild dysplasia or grade 1 cervical intraepithelial neoplasia (CIN) [4–6], have generated considerable interest in the possible role of human papillomavirus (HPV) in genital tract cancers. Prospective epidemiologic studies have shown that CIN lesions have the potential to progress to invasive cancer [7].

Papillomaviruses have an oncogenic potential in animal models as well as in humans. Bovine papillomaviruses are implicated in both naturally occurring and experimentally produced cancers [8–10]. In humans, malignant transformation of lesions of epidermodysplasia verruciformis is well documented

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Abbreviations:
CIN: cervical intraepithelial neoplasia
HPV: human papillomavirus

[11,12] and such transformation is also reported for condyloma acuminatum [13]. Some authors have emphasized the possible role on HPV in squamous cell carcinoma of the cervix [14,15]. Association of condyloma with dysplasia is not uncommon [16–18].

Marked cytoplasmic vacuolization of superficial squamous cells with atypical, eccentric, pyknotic nuclei (koilocytotic atypia) is the characteristic feature of papillomavirus infection but tissues histologically diagnosed as condylomas often display associated changes of intraepithelial neoplasia within the same field [18]. In a previous investigation, we examined 97 biopsies of histologically confirmed condylomatous lesions of the cervix and found both virus particles and viral antigen in 24 (25%) and only viral antigen in 23 (23%) [19]. In the present report, we have further analyzed the cytohistologic features of these condylomas with respect to the presence of associated CIN to determine whether or not the prevalence of virus antigen varies with the histologic appearance of the lesion.

MATERIALS AND METHODS

Tissues

These were punch biopsies of flat or papillary condylomatous lesions of the cervix in women seen at the Colposcopy Clinic of the Sir Mortimer B. Davis Jewish General Hospital, Montreal. The women were 16–35 years old and were seen over a 16-month period. All of the tissues were histologically diagnosed as condylomas.

Tests for Viral Antigen

The immunologic studies of these tissues for viral antigen have been described [19]. There are many different types of HPV that are characterized by different nucleotide sequences and different capsid protein antigens; we used a broadly cross-reactive rabbit antiserum which is capable of reacting with capsid antigen of all human papillomavirus types [20]. In brief, paraffin-embedded biopsies were sectioned at 5–6 μm and were stained by the peroxidase-antiperoxidase technique of Sternberger [21]. Specific staining was seen as dark brown granular reaction product localized in the nuclei of superficial epithelial cells (Fig 1).

Histopathology

Adequate histologic sections were available for 94 of the 97 cervical condylomas previously studied. The lesions were classified histologically into 2 groups regardless of their growth pattern and without knowledge of the results of the immunologic tests: (1) flat condyloma (planum), papillary condyloma (acuminatum), or endophytic condyloma; (2) ordinary condyloma and CIN with condylomatous (koilocytotic) features. The first group contained koilocytes particularly in the upper epithelial strata. These cells were characterized by perinuclear cytoplasmic cavitation (ballooning) and nuclear enlargement, hyperchromasia, degeneration, pyknosis, and karyorrhexis. Multinucleated and dyskeratotic cells (individual cell keratosis) were often seen. The parabasal and basal cells were arranged in an orderly fashion with normal nucleocytoplasmic ratio and evenly distributed chromatin (Fig 2). Mitotic figures, when present, were normal, although occasional tripolar forms were observed. These are often encountered in virus-infected cells as well as regenerative cells in general, and reflect nuclear polyploidy. The second group contained, in addition to koilocytic features, poorly differentiated basal and parabasal cells consistent with intraepithelial neoplasia. This implied that a certain proportion of these cells' nuclei had an aneuploid DNA content [22]. Also, the cohesion and organization of these cells were disturbed; they had high nucleocytoplasmic ratio, irregular nuclear size and shape, coarse chromatin, and abnormal mitotic figures. According to the extent of epithelial involvement, the lesions were divided into CIN grade 1 (mild dysplasia) and CIN grade 2 (moderate dysplasia). In the former, abnormal cells with nuclear aneuploidy were confined to the lower one-third of the epithelium, whereas in the latter the lower one-half of the epithelium was composed of koilocytes with perinuclear cytoplasmic ballooning similar to that in ordinary condylomata. However, the nuclei of koilocytes associated with CIN lesions were larger, more irregular, and hyperchromatic as compared to koilocytes in ordinary condylomata (Figs 3,4).

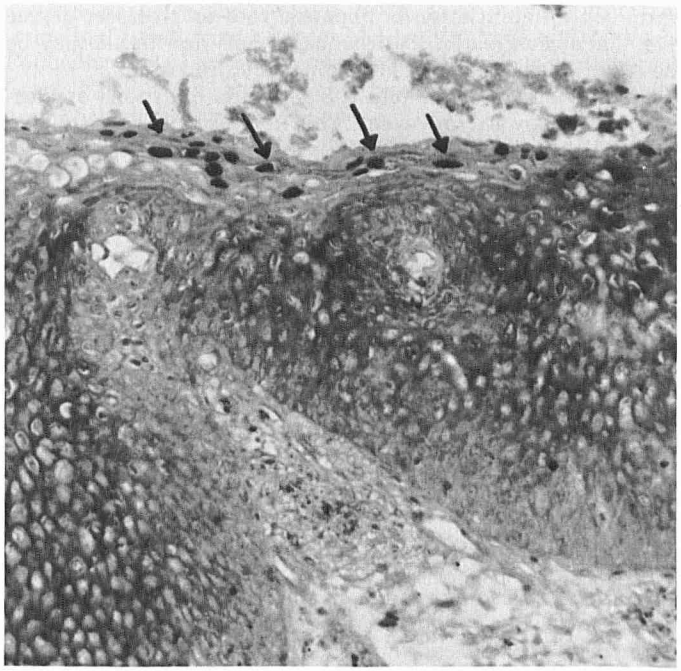

FIG 1. Darkly staining nuclei (*arrows*) of koilocytotic cells of flat condyloma represent immunoreactivity to HPV antigen (× 380).

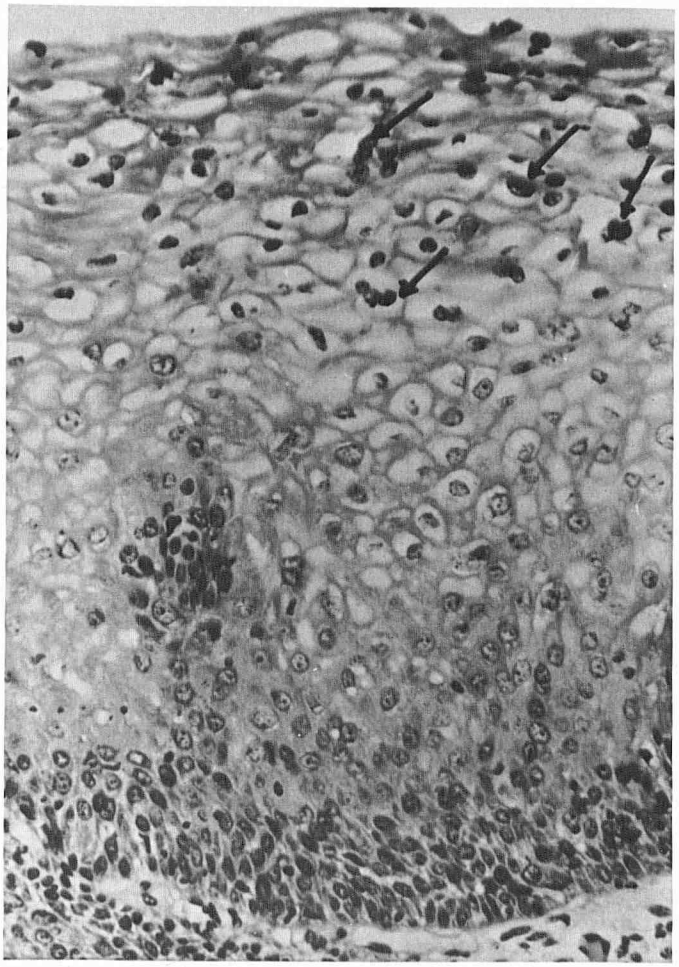

FIG 2. Flat condyloma (planum) of cervix. Koilocytotic cells with cytoplasmic ballooning and enlarged degenerative (*arrows*) and often double nuclei occupy the upper third of the epithelium. The basal and parabasal layers contain normal to slightly reactive cells (× 100).

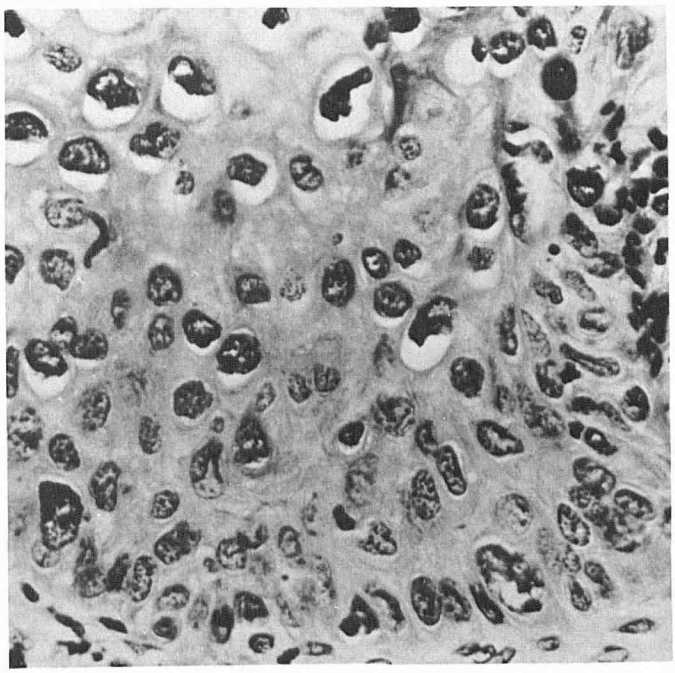

FIG 3. Cervical intraepithelial neoplasia grade 1 (mild dysplasia) with condylomatous features. The lower ⅓ of the epithelium contains atypical, pleomorphic nuclei displaying loss of cohesion and organization, and coarse, often aggregated chromatin. Such cells are believed to have aneuploid nuclear DNA content. The upper ⅔ to ½ of the epithelium has koilocytotic cells, many of which contain nuclear degenerative changes (× 250).

RESULTS

Viral antigen was present only in the nuclei of vacuolated or flattened, eosinophilic cells of the superficial epithelium and was found only in a proportion of cells displaying viral cytopathology. The prevalence of viral antigen varied with the histologic type of the lesion. The proportion of tissues positive for antigen decreased as the features of associated dysplasia became more marked (Table I). Of the 51 tissues that were classified as ordinary condylomas, 42 (82%) were positive for antigen. Eighteen tissues were classified condylomas with grade 2 CIN; none of these was antigen-positive. Of the 25 lesions in the intermediate category, viz, condylomas with grade 1 CIN, 8 (32%) were antigen-positive. In 6 of these 8, the distribution of antigen was similar to that in the ordinary condylomas but in the remaining 2, only a few scattered cells were found to be antigen-positive.

DISCUSSION

In this series of histologically confirmed condylomas of the cervix, almost one-half of the tissues contained, in addition to koilocytotic atypia suggestive of HPV infection, grade 1–2 CIN (mild to moderate dysplasia) within the same microscopic field. A high proportion of the tissues that displayed purely condylomatous changes was positive for viral antigen. The 82% rate of antigen prevalence in this group is as high as, or higher than, that in warts at any site on the body. It is clear that this group represents simple productive papillomavirus infection with characteristic virally induced cytopathology.

The antigen prevalence decreased markedly as the features of associated intraepithelial neoplasia became more severe; none of the 18 condylomas with grade 2 CIN features was positive for viral antigen. The presence of viral capsid antigen, as detected by the immunologic test employed, is indicative of productive virus infection. The absence of evidence of productive virus infection in grade 2 CIN with condylomatous features does not rule out a papillomavirus etiology for these CIN lesions; we have to consider that the papovavirus-transformed cells, while they continue to harbor the viral genome, do not synthesize viral particles. Several alternative relationships between virus infection and CIN are possible: (1) The CIN is a result of viral transformation of cells in the course of a papillomavirus infection. (2) The CIN is unrelated to papillomavirus infection; it merely coexists side by side with the virus infection or the neoplastic epithelium is secondarily infected with the

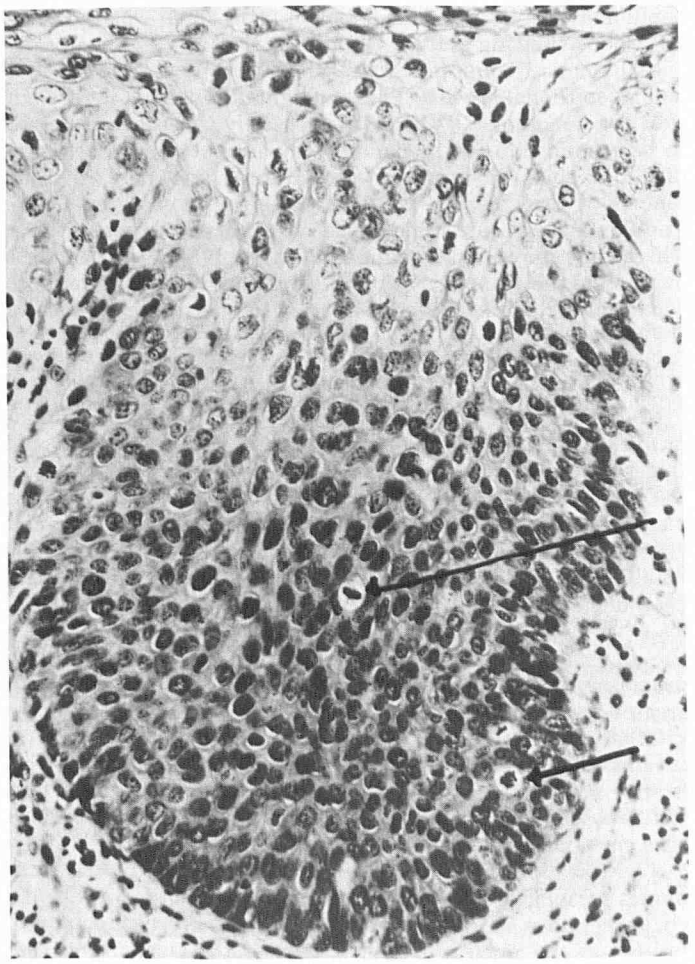

FIG 4. Cervical intraepithelial neoplasia, grade 2 (moderate dysplasia) with condylomatous features. The lower ½ of the epithelium is replaced by abnormal cells with pleomorphic, hyperchromatic nuclei. In addition to loss of cellular organization and cohesion and maturation, there are several mitotic figures (*arrows*). The upper ½ of the epithelium demonstrates attempt at cytoplasmic maturation, although the nuclear morphology remains abnormal. The most superficial cells have irregular cytoplasmic cavities and dark, degenerative nuclei consistent with koilocytotic atypia associated with presence of HPV (× 150).

TABLE I. *Prevalence of virus antigen by histologic characteristics of cervical lesions with condylomatous features*

Category	Number tested	Number with antigen (%)
Ordinary condyloma	51	42 (82%)
CIN grade 1 with condylomatous features (mild dysplasia)	25	8 (32%)
CIN grade 2 with condylomatous features (moderate dysplasia)	18	0 (0%)
	94	50

CIN = cervical intraepithelial neoplasia.

papillomavirus. (3) The diagnosis of condyloma was incorrect for lesions showing grade 2 CIN.

In humans, papillomaviruses are generally responsible for benign epithelial growth. Their malignant potential is rare but well documented; HPV type 5, especially, has been reported to be responsible for malignant transformation of flat warts [22,23] in epidermodysplasia verruciformis, and there are some reports of rare invasive and metastasizing condylomas [13]. Papillomaviruses are widespread in different animal species where they may induce malignant tumors [8–10]. In Shope's papilloma, the transformation of virus-induced papilloma into carcinoma is classically correlated with the disappearance of the virions. Therefore, it is possible that the disappearance of viral antigen in condylomas with dysplasia is a comparable phenomenon correlated with cell transformation.

In this respect, Bowenoid papules might raise a similar problem. The histopathologic features of Bowenoid papules of the genitalia are very comparable to condylomatous atypias of the cervix; their prognosis is not that of a true Bowen's disease but is considered uncertain. The comparison between condylomas with dysplasia and Bowenoid papules deserves to be emphasized since the presence of human papillomavirus has been demonstrated occasionally in lesions of Bowenoid papulosis [24–28]. Since papillomavirus particles are synthesized in the upper layers of the epidermis, it is not surprising to find progressively less synthesis of virus particles in lesions with greater atypia.

Other authors failed to demonstrate capsidal antigens in several cutaneous tumors and verrucous carcinomas [25]. This is why virologists look for persistence of naked viral DNA (undetectible with PAP staining that demonstrates only virions with capsidal antigens) by the techniques of molecular biology: DNA extraction from total viral DNA and typing by the techniques of nucleic acid hybridization. The studies of Fu and colleagues have shown that CIN lesions are heterogeneous with respect to the nuclear DNA content of their cells, and that aneuploid lesions are more likely to progress to invasive cancer than grade 1, well-differentiated diploid or polyploid CIN [26]. Papillomavirus antigen is found frequently in the early form of CIN which is diploid or polyploid, but rarely in aneuploid lesions [27,28]. However, a number of recent reports have documented the presence of genital tract papillomavirus genomes in carcinoma in situ and invasive cancer of the cervix [29–31].

Taken together, these findings suggest that papillomavirus infection of the genital tract is an important factor in the development of cervical cancer.

REFERENCES

1. Kessler JJ: Human cervical cancer as a venereal disease. Cancer Res 36:783–791, 1976
2. Underwood PB, Hester LL: Diagnosis plus treatment of premalignant lesions of the vulva: a review. Am J Obstet Gynecol 110:849–857, 1971
3. Rhatigan RM, Jimenez S, Chopskie EJ: Condyloma acuminatum and carcinoma of the penis. South Med J 65:423–428, 1977
4. Meisels A, Fortin R: Condylomatous lesions of the cervix and vagina. I. Cytologic patterns. Acta Cytol (Baltimore) 20:505–509, 1976
5. Purola E, Savia E: Cytology of gynecologic condyloma acuminata. Acta Cytol (Baltimore) 21:26–31, 1977
6. Laverty CR, Russell P, Hills E, Booth N: The significance of the non condylomatous wart virus infection of the cervical transformation zone. A review with two illustrative cases. Acta Cytol (Baltimore) 22:195–201, 1978
7. Richart RM, Barron BA: A follow up study of patients with cervical dysplasia. Am J Obstet Gynecol 105:386–393, 1969
8. Jarett W, McNeil P, Grimshaw W, Selman I, Mc Intyre W: High incidence of cattle cancer with a possible interaction between an environmental carcinogen and a papillomavirus. Nature 274:215–217, 1978
9. Lowy D, Dvoretzky I, Shovert R, Law M, Engel L, Howley P: In vitro tumorigenic transformation by a defined subgenomic fragment of bovine papillomavirus DNA. Nature 287:72–74, 1980
10. Olson C, Gordon D, Roby M, Lee K: Oncogenicity of bovine papillomavirus. Arch Environ Health 19:827–837, 1969
11. Jablonska S, Dabrowski J, Jabukowicz K: Epidermodysplasia verruciformis as a model in studies of the role of papovavirus in oncogenesis. Cancer Res 32:583–589, 1972
12. Reuter M, Van Mullen P: Behavior of virus in malignant degeneration of skin lesions in epidermodysplasia verruciformis. J Invest Dermatol 54:324–333, 1970
13. Kovi J, Tillman R, Lee S: Malignant transformation of condyloma acuminatum. Am J Clin Pathol 61:702–710, 1974
14. Orth G, Breitburd F, Faure M, Croissant O: Papillomaviruses: possible role in human cancer, Cold Spring Harbor Conferences on Cell Proliferation, vol 4, Origins of Human Cancer. Edited by HH Hiatt, JD Watson, JA Wisten. New York, Cold Spring Harbor, 1977, p 1043
15. Zur Hausen H: Human papillomavirus and their possible role in squamous cell carcinomas. Curr Top Microbiol Immunol 78:1–31, 1977
16. Guillet G, Gupta P, Braun L, Shah K: Condylomes plans dysplasiants du col: un modèle pour l'étude des premières étapes de la carcinogénèse virale. J Gynecol Obstet Biol Reprod (Paris) 11:249–253, 1982
17. Guillet G, Braun L, Gupta P, Shah K, Ferenczy A: Condylomes plans et dysplasies cervicales. Ann Dermatol Venereol 110:43–51, 1983
18. Meisels A, Morin C: Human papillomavirus and cancer of the uterine cervix. Gynecol Oncol 12:5111–5123, 1981
19. Ferenczy A, Braun L, Shah KV: Human papillomavirus (HPV) in condylomatous lesions of the cervix: a comparative ultrastructural and immunohistochemical study. Am J Surg Pathol 5:661–670, 1981
20. Jenson AB, Rosenthal JR, Olson C, Pass F, Lancaster WD, Shah KV: Immunological relatedness of papillomaviruses from different species. JNCI 64:495–496, 1980
21. Sternberger LA: Immunocytochemistry, Englewood Cliffs, NJ, Prentice Hall, 1974
22. Lutzner MA: Epidermodysplasia verruciformis: an autosomal recessive disease characterized by viral warts and skin cancer. A model for viral oncogenesis. Bull Cancer 2:169–182, 1978
23. Lutzner MA, Croissant O, Ducasse M-F, Kreis H, Crosnier J, Orth G: A potentially oncogenic human papillomavirus (HPV-5) found in two renal allograft recipients. J Invest Dermatol 75:353–356, 1980
24. Guillet G, Braun L, Aftimos J, Geniaux M, Texier L: Bowenoid-papulosis: demonstration of human papillomavirus (HPV) with anti-HPV immune-serum. Arch Dermatol, in press
25. Farmer ER, Braun L, Shah KV: Immunologic staining for human papillomavirus in cutaneous dysplasia (abstr). Arch Dermatol 116:1389, 1980
26. Fu YS, Reagan JW, Richart RM: Definition of precursors. Gynecol Oncol 12:5220–5231, 1981
27. Crum CP, Braun LA, Shah KV, Fu YS, Levine RU, Fenoglio CM, Richart RM, Townsend DE: Vulvar intraepithelial neoplasia: correlation of nuclear DNA content and the presence of a human papillomavirus (HPV) structural antigen. Cancer 49:468–471, 1982
28. Fu YS, Braun L, Shah KV, Lawrence WD, Robboy SJ: Histologic nucleic DNA and human papillomavirus studies of cervical condylomas. Cancer, in press
29. Zachow KR, Ostrow RS, Bender M, Watts S, Okagaki T, Pass F, Faras AJ: Detection of human papillomavirus DNA in anogenital neoplasias. Nature 300:771–773, 1982
30. Gissman L, Wolnik L, Ikenberg H, Koldovsky U, Schnurch HG, Zur Hausen H: Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. Proc Natl Acad Sci USA 80:560–563, 1983
31. Durst M, Gissman L, Ikenberg H, Zur Hausen H: A papillomavirus from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci USA, in press, 1983